Proposal to reclassify Propionibacterium acnes type I as Propionibacterium acnes subsp. acnes subsp. nov. and Propionibacterium acnes type II as Propionibacterium acnes subsp. defendens subsp. nov

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Running title:

Taxonomic reclassification of *P. acnes* types I and II

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Abstract

Recently, strains of *Propionibacterium acnes* from the type III genetic division have been proposed as *Propionibacterium acnes* subsp. *elongatum* subsp. nov., with strains from the type I and II divisions collectively classified as *Propionibacterium acnes* subsp. *acnes* subsp. nov. Under such a taxonomic re-appraisal, we believe that types I and II should also have their own separate rank of subspecies. In support of this, we describe a polyphasic taxonomic study based on the analysis of publically available multilocus and whole genome sequence datasets, alongside a systematic review of previously published phylogenetic, genomic, phenotypic and clinical data. Strains of types I and II form highly distinct clades based on multilocus sequence analysis (MLSA) and whole genome phylogenetic reconstructions. *In silico* or digital DNA-DNA similarity values also fall within the 70-80% boundary recommended for bacterial subspecies. Furthermore, we see important differences in genome content, including the presence of an active CRISPR/Cas system in type II strains, but not type I, and evidence for increasing linkage equilibrium within the separate divisions. Key biochemical differences include positive tests for β-haemolytic, neuraminidase and sorbitol fermentation activities with type I strains, but not type II. We now propose type I strains as *Propionibacterium acnes* subsp. *acnes* subsp. nov., and type II as *Propionibacterium acnes* subsp. *defendens* subsp. nov. The type strain of *Propionibacterium acnes* subsp. *acnes* subsp. nov. is NCTC 737T (=ATCC 6919T=JCM 6425T=DSM 1897T=CCUG 1794T), while the type strain of *Propionibacterium acnes* subsp. *defendens* subsp. nov. is ATCC 11828 (=JCM 6473=CCUG 6369).

Propionibacterium acnes is a Gram-positive anaerobic bacterium and a member of the 'cutaneous' group of human propionibacteria along with *Propionibacterium granulosum*, *Propionibacterium avidum* and *Propionibacterium humerusii.* Although found predominately on the skin, it can also be isolated from the oral cavity and the genitourinary and gastrointestinal tracts (Patrick and McDowell, 2011). While the bacterium is most noted for its association with the inflammatory skin condition acne vulgaris (Lomholt and Kilian, 2010, McDowell et al., 2012, Fitz-Gibbon et al., 2013), there is now a growing recognition that the spectrum of opportunistic infections and clinical conditions to which it may be associated has been underestimated (Tunney et al., 1999, Cohen et al., 2005, Cavalcanti et al., 2011, Eishi, 2013; Barnard et al., 2016).

In the last 10 years, significant advances in our understanding of this bacterium at the population genetic level have been made using single, multilocus and whole genome sequence analyses (McDowell et al., 2005, McDowell et al., 2008, Lomholt and Kilian, 2010, McDowell et al., 2012, Fitz-Gibbon et al., 2013, Tomida et al., 2013, Scholz et al., 2014). Such work has demonstrated the phylogenetically distinct nature of the originally described *P. acnes* serotypes, designated types I and II, and identified a new type, designated type III, which displays an ability to form long filamentous cell structures not seen with types I and II (McDowell et al., 2005, McDowell et al., 2008). These studies have also identified further phylogenetic subdivisions within the type I clade (IA_1, IA_2, IB, IC) which differ in genome content, inflammatory potential, association with disease, production of putative virulence determinants, resistance to antibiotics used in the treatment of acne, as well as biochemical and aggregative properties (Valanne et al., 2005, McDowell et al., 2013, Tomida et al., 2013, Johnson et al., 2016, Scholz et al., 2016).

Very recently, Dekio *et al*. (2015) proposed that *P. acnes* type III be reclassified as *Propionibacterium acnes* subsp. *elongatum* subsp. nov. based on phylogenetic, genomic and phenotypic differences, with strains of type I and II classified as *Propionibacterium acnes* subsp. *acnes* subsp. nov. (Dekio et al., 2015). In bacterial taxonomy, there are currently no clear guidelines for the establishment of subspecies, and the proposal of such essentially remains at the discretion of the researcher. Nevertheless, the proposal of a new bacterial subspecies is normally based on consistent phylogenetic differences and phenotypic variations between groups of strains within a species (Brenner et al., 2000). If the major phylogroups of *P. acnes* are now to be reclassified within a subspecies framework, then strains of types I and II also deserve their own taxonomic rank of subspecies. In this paper, we describe a polyphasic taxonomic study based on the analysis of publically available multilocus sequence and whole genome datasets, alongside a review of published phylogenetic, genomic, phenotypic and clinical data, to support the reclassification of *P. acnes* types I and II as distinct subspecies. We propose type I as *Propionibacterium acnes* subsp. *acnes* subsp. nov. as it contains the type strain (ATCC6919), and type II as *Propionibacterium acnes* subsp. *defendens* subsp. nov. Type III strains remain as *Propionibacterium acnes* subsp. *elongatum* subsp. nov. (hereafter described as type III) as previously proposed (Dekio et al., 2015).

In 2005, we demonstrated that the *P. acnes* serotypes known as types I and II represented highly distinct phylogenetic groups based on sequence analysis of the *recA* housekeeping gene, as well as the putative haemolysin/ FtsJ-like methyltransferase gene *tly* (McDowell et al., 2005). Application of *recA* typing was also central in the identification of strains representing the type III phylogenetic division (McDowell et al., 2008). Since then, two key MLSA methods based on eight ($MLSA_8$) and nine protein-encoding genes ($MLSA_9$) have been

described for this bacterium, both based on completely different sets of genetic loci (Lomholt and Kilian, 2010, McDowell et al., 2012). With both independent MLSA schemes we find that types I, II and III form highly distinct clades consistent with the original *recA* and *tly* analysis, and supported by high bootstrap values (Fig. 1). This phylogenetic clustering is also highly congruent with that obtained upon whole genome analysis of 124,731 SNPs in shared or 'core' regions of 85 *P. acnes* genomes spanning all the major phylogenetic divisions (Fig. S1); the average p-distance between each of the types based on core region analysis is 0.444 for types I and II, 0.487 for types I and III, and 0.470 for types II and III (Table 1). Twenty six percent of core region SNPs are unique to type I, with 22% unique to type II and 24% unique to type III (Table 1). The genetic distance between types I and II is therefore similar to the distance between type I and type III, and type II and type III. In addition, even though the 16S rRNA gene of *P. acnes* demonstrates a high degree of intra-species sequence identity, the observation of distinct and non-overlapping ribotypes for type I (RT1; RT3; RT4; RT5; RT8; RT16; RT532), type II (RT2; RT6), and type III (RT9) provides further evidence for their different phylogenies (Fitz-Gibbon et al., 2013, Barnard et al., 2016) (Fig S1).

Alongside phylogenetic analyses, previous whole genome typing patterns based on methods such as Random Amplification of Polymorphic DNA (RAPD) and noncoding repeat sequences, as well as the analysis of non-core regions, also support types I and II as highly distinct divisions at the genome level (Perry et al., 2003, Tomida et al., 2013, Hauck et al., 2015). While digital or *in silico* DNA-DNA hybridization values (GGDC 2.0 algorithm) between types I, II and III are above the 70% cut-off value currently used for bacterial species demarcation, thus confirming their membership of the same species, the whole genome relatedness values are consistent with the proposal that types I and II are also placed in distinct taxonomic ranks

116 in line with that recently proposed for type III (Dekio et al., 2015). Strains representing the
117 different phylogroups within type I (IA_1, IA_2, IB, IC) share high *in silico* DNA-DNA hybridization
118 values of 91-100%, but this drops to 74.1-78.5% when analysed against the type II strains
119 ATCC11828 and JCM18920, and 72.0-72.8% with the type III strain JCM18909 (Dekio et al.,
120 2015). Strains of type II and III share relatedness values of 72.9-73.2% (Dekio et al., 2015).
121 These hybridization values between the major divisions are within the 70-80% similarity
122 boundary recently recommended for bacterial subspecies (Meier-Kolthoff et al., 2014).

123 Detailed comparative analysis of type I and II whole genome sequences also reveals some
124 salient differences between the divisions. These include specific genomic inversions and
125 insertions present in type II strains, but not type I, which encode genes related to
126 carbohydrate processing and modification, ABC transporters, nickel import, bacitracin
127 resistance and hypothetical proteins (Fig. S2) (McDowell et al., 2013, Scholz et al., 2016). One
128 of the most striking differences relates to the presence in type II strains of clustered regularly
129 interspaced short palindromic repeats (CRISPR)/Cas locus (Brüggemann et al., 2012b, Fitz-
130 Gibbon et al., 2013). In contrast, type I and type III strains contain CRISPR/Cas gene remnants
131 within their genome, indicating deletion of the locus during the evolutionary history of these
132 phylogroups; the deletions are more extensive in type I strains compared to type III. The
133 deletion of the CRISPR/Cas system in type I and type III strains makes these divisions more
134 susceptible to horizontal gene transfer (HGT) and the acquisition of fitness or virulence traits.
135 The observation of such CRISPR/Cas gene remnants has led to the suggestion that the type I
136 and III divisions may constitute younger subpopulations than type II strains which are
137 descended from a more ancient lineage (Brüggemann et al., 2012a, Brüggemann et al.,
138 2012b). Since age = *ds*/(clock rate x 2), where *ds* is the mean number of synonymous

substitutions per site and clock rate is the synonymous molecular clock rate, calculation of the *ds* values for strains currently representing the major type I, II and III divisions may give deeper insights into their relative ages. Interestingly, using the Nei-Gojobori method (Jukes-Cantor) (Nei and Gojobori, 1986) in MEGA v7.0 (Kumar et al., 2016), we observed that the *ds* value for the entire type I division was slightly higher than type II based on an initial analysis of concatenated $MLSA_8$ sequence data, while type III values were lower (Table 1). To investigate this further, we examined the shared core-coding regions of 85 *P. acnes* genomes currently available. Multiple sequence alignments were performed using MUSCLE (Edgar, 2004) and the Jukes-Cantor *ds* values calculated for each pair of sequences in the alignment using the Nei-Gojobori method as implemented in the Bioperl package Bio::Align::DNAStatistics (Stajich, 2002). As before, the resulting *ds* values obtained for type I (0.008), type II (0.005) and type III (0.001) revealed higher synonymous nucleotide diversity within the large type I clade compared to type II and type III, indicative of an older age. Further studies are therefore required to provide clarity on the series of evolutionary events that have given rise to the emergence and diversity of the current *P. acnes* clades now proposed as subspecies, including the possible diversity-purging effects of periodic selection (Cohan, 2001).

Propionibacterium acnes has a clonal, epidemic population structure and is in linkage disequilibrium, though rates of HGT within the population as a whole are statistically significant (Lomholt and Kilian, 2010, McDowell et al., 2012, McDowell et al., 2013). Previous studies have, however, found that rates of recombination appear to differ throughout the population, and that the association of alleles is less significant when distinct phylogroup populations are considered (McDowell et al., 2012, McDowell et al., 2013). In particular, we

see a drop in the index of association value (I_A) when strains from the type I and II divisions are considered separately, indicating increasing linkage equilibrium within these distinct clusters (McDowell et al., 2013); this can also be observed on a Neighbour-Net split graph based on $MLST_8$ allelic profile data (Fig. 2). Detailed inspection of $MLSA_8$ datasets also suggests conjugal transfer and replacement of unusually large chromosomal segments in the genome dynamics of the type I clade, particularly between types IA_2 and IB (Lomholt and Kilian, 2010, McDowell et al., 2012, McDowell et al., 2013). The idea that rates of genetic interchange are more frequent within, but not between, the major divisions suggests increasing sexual isolation which occurs with more genetically divergent organisms (Majewski, 2001). Reduced rates of recombination may also indicate ecological differences since members of the same habitat are more likely to undergo recombination events (sympatric speciation); such population subdivisions can introduce linkage disequilibrium into an analysis if isolates from different niches (Ecotypes) are included (Spratt and Maiden, 1999). Comprehensive analysis of genome differences between the major types does indeed provide potential evidence for distinct environmental challenges within the human host.

Studies by Johnson and Cummins (1972) first revealed types I and II as distinct phenotypes of *P. acnes* based on serological agglutination tests and cell wall sugar analysis; type I strains contain galactose in their cell wall, but this sugar is absent in type II strains which occasionally also contain *meso*-Diaminopimelic acid (DAP) (Table 2). The development of more recent monoclonal antibody typing methods for *P. acnes* have further highlighted differences between the cell wall structures of type I and II, as well as type III, based on the expression of unique antigenic determinants, including those in lipoteichoic acid and adhesin proteins (Holland et al., 2010, McDowell et al., 2011, Bae et al., 2014). Differences in cell surface

hydrophobicity have also been described for types I and II, and upon growth in liquid media, such as protease peptone yeast (PPY) or brain heart infusion (BHI) broth, type II strains form a turbid solution with a slight fine sediment, while strains of type IA and IC can form a large granular sediment or auto-aggregate with a clear solution (Cohen et al., 2005); type IB strains behave as type II in respect to this characteristic. Types I and II can be differentiated from one another and type III based on the analysis of bacterial whole cell proteins by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) fingerprinting, highlighting further variation at the phenotype level (Nagy et al., 2013, Dekio et al., 2015). Furthermore, differences in the susceptibility of types I and II to bacteriophage infection have also been known for some time (Webster and Cummins, 1978, Liu et al., 2015). The main phylogroups of *P. acnes* share a high degree of similarity with regard to their biochemical phenotype, including traditional tests used to differentiate the bacterium from other 'cutaneous' propionibacteria (Table 2). Notable phylogroup differences, however, include β-haemolytic and neuraminidase activity, as well as sorbitol fermentation, all of which are essentially restricted to the type I division (McDowell et al., 2008, Lomholt and Kilian, 2010, Niazi et al., 2010) (Table 2). The production of lipase also appears much lower amongst type II strains versus those from the type I and III divisions (McDowell et al., 2008, Niazi et al., 2010) (Table 2); we previously described how type II strains have deletions in the TATA box and open reading frame of two candidate lipase genes which may explain this reduced activity (Tomida et al., 2013).

One defining difference between the type I and II phylogroups rests on their association with acne vulgaris. On the basis of both culture and metagenomic analyses, widely disseminated clonal lineages from the type I division have been described in association with acneic skin,

208 but not those from the type II or type III divisions which appear to be associated more with
209 blood, medical device and soft tissue infections (Lomholt and Kilian, 2010, McDowell et al.,
210 2011, McDowell et al., 2012, Fitz-Gibbon et al., 2013, Rollason et al., 2013). Recently, type III
211 strains have also been linked with the depigmenting skin condition progressive macular
212 hypomelanosis (Peterson et al., 2015, Barnard et al., 2016). Interrogation of the *P. acnes*
213 $MLST_8$ isolate database, which contains information on a large collection of geographically
214 widespread isolates and their clinical source, reveals a statistically significant enrichment
215 overall for strains from the type I clade in acneic versus healthy skin ($p<0.001$; Fishers exact
216 test, two tailed), while those from the type II clade appear to show no association overall
217 ($p=0.213$; Fishers exact test). More specifically, associations are found between acneic skin
218 and strains from the type IA_1 clonal complexes CC1 (RT1 and RT532) ($p<0.01$; Fishers exact
219 test), CC3 (RT1, RT4 and RT5) ($p=0.043$; Fishers exact test) and CC4 (RT8) ($p=0.021$; Fishers
220 exact test) (Fig 1 and S1). In a previous study, we found that a globally disseminated clonal
221 lineage with the MLST genotype ST6 (Warwick $MLST_7$ scheme analysis) or ST1 ($MLST_8$ analysis)
222 strikingly represented the majority of type IA_1 isolates we analysed from a cohort of patients
223 with acne (McDowell et al., 2011). In contrast, specific type II lineages (RT2 and RT6) belonging
224 to CC72 ($MLST_8$) appear associated with healthy skin based on metagenomic and culture-
225 based detection (McDowell et al., 2012, Fitz-Gibbon et al., 2013, Johnson et al., 2016). The
226 observation that type II strains, but not those from the type I clade, encode CRISPR/Cas
227 elements may be important in this context, thus preventing the acquisition of genetic loci that
228 may contribute to virulence and acne pathophysiology (Fitz-Gibbon et al., 2013). For example,
229 key type I lineages from CC3 ($MLST_8$; Fig. 1), believed to be associated with acne contain a
230 novel plasmid with a tight adhesion (Tad) locus and two unique genomic islands, known as
231 loci 1 and 2, that contain genes proposed to enhance virulence via increased bacterial

adhesion and host immune response (Fitz-Gibbon et al., 2013, Tomida et al., 2013, Kasimatis et al., 2013).

To conclude, we now propose *P. acnes* type I and II as distinct subspecies based on a polyphasic taxonomy approach. The growing number of genomes now becoming available for other propionibacteria will also provide an important opportunity to reexamine the genus and the place of the 'cutaneous' group within it.

Description of *Propionibacterium acnes* subsp. *acnes* subsp. nov.

Propionibacterium acnes subsp. *acnes (*ac'nes Gr. n. *acme* a point; incorrectly transliterated as N.L. n. *acne* acne; N.L. gen.n. *acnes* of acne). Description based on McDowell et al. (2008), Niazi et al. (2010), Patrick and McDowell (2011), and Dekio et al. (2015).

Four phylogenetically distinct type I groups have been described, known as type IA_1, IA_2, IB and IC; type IA_2, IB and IC represent phylogenetically tight clusters compared to IA_1. Cells are Gram-positive, nonmotile, non-spore forming, and anaerobic-to-aerotolerant. Colonies appear as lenticular, minute-to-4.0 mm, white, can become tanned, pink or orange in 3 weeks. Growth is most rapid at 30-37°C. Surface colonies on blood agar (horse or rabbit) are punctiform-to-0.5 mm, circular, entire-to-pulvinate, translucent-to-opaque, white-to-gray, glistening. The cell shape after anaerobic culture in broth medium ranges from small plump rods to ellipsoids which tend to occur in pairs joined at a slight angle, and the size is approximately 0.4-to-0.5 by 0.8-to-0.9 µm. In defined medium broth culture, type IA and IC strains form a turbid suspension, while in PPY or BHI broth they form a settled granular sediment with a clear solution. In contrast, type IB strains form a slight fine sediment and turbid solution containing suspended cells. In suitable media with good growth, the final pH is 4.5-5.0. Generally catalase positive, cultures need to be exposed to air for 1 h before testing.

All strains have an absolute requirement for pantothenate, while thiamine, biotin and nicotinamide are stimulatory. Strains are co-haemolytic and variable for β-haemolytic activity and produce a number of extracellular enzymes including ribonuclease, neuraminidase, hyaluronidase, acid phosphatase, lecithinase and lipase. Strains of type IA produce relatively low levels of the putative co-haemolytic Christie-Atkins-Munch-Peterson (CAMP) factor 1, but type IB strains produce an abundance of this protein. The total quantity of acid (especially the proportion of lactic acid) produced from fermentable carbohydrates is highly variable. Cells ferment glucose, but not sucrose or maltose. Lactate is converted to propionate by most strains but only if the initial oxidation-reduction potential of the medium is sufficiently low, or if the initial growth rate is rapid. Sorbitol fermentation is a variable but defining characteristic of type I strains. Gelatin is hydrolysed, and most strains produce indole and reduce nitrate, but esculin is not hydrolysed. The major long chain fatty acid produced in thioglycolate cultures is 13-methyltetradecanoic acid (32-62%) and iso-C15:0 FAME is the predominant cellular fatty acid. Prominent mass ions obtained by MALDI-TOF mass spectrometry are at 3,589 Da and 7,179 Da. Peptidoglycan contains alanine, glutamic acid, glycine and LL-DAP. Cell wall sugars are glucose, mannose and galactose. Strains have been isolated from the human skin, oral cavity and genitourinary tract. Type IA_1 and IC strains are associated with acne vulgaris. The G+C content is ~ 60% based on whole genome sequencing analysis.

This subspecies is the type subspecies of *P. acnes* and contains the type strain according to Rules 40a and 40b of the Bacteriological Code (Lapage et al., 1992). The type strain is NCTC 737T (=ATCC 6919T=JCM 6425T=DSM 1897T=CCUG 1794T), original type strain of the species, isolated from facial acne in London, 1920 (Genbank accession number NZ_JNHS00000000).

Description of *Propionibacterium acnes* subsp. *defendens* subsp. nov.

Propionibacterium acnes subsp. *defendens* (de.fen'dens L. part. adj. *defendens,* defending, guarding, protecting; referring to the fact that strains have an active CRISPR/Cas system which guards or controls against foreign mobile genetic elements). Description based on McDowell et al. (2008), Niazi et al. (2010), Patrick and McDowell (2011), and Dekio et al. (2015).

Cells are Gram-positive, nonmotile, non-spore forming, and anaerobic-to-aerotolerant. Their cellular and colony morphology is similar to type I cells, but they may appear more coccoid and are most similar to previous descriptions for 'Corynebacterium parvum' which is a synonym for *P. acnes*. In defined medium broth culture, strains form a slight fine sediment and turbid solution containing suspended cells. In addition to pantothenate, some strains require haem and vitamin K to grow. Biochemical phenotype is similar to type I strains but with some notable differences. Cells are negative for β-haemolysis, and neuraminidase and lipase activity is infrequently found. Abundant levels of CAMP factor 1 are produced; similar to that observed with strains of type IB. Sorbitol fermentation is negative. The predominant cellular fatty acid is iso-C15:0 FAME and prominent mass ions obtained by MALDI-TOF mass spectrometry are 3,628 Da and 7,258 Da. Peptidoglycan contains alanine, glutamic acid, glycine, LL-DAP, and occasionally *meso*-DAP. Cell wall sugars are mannose and glucose, but galactose is not present. Strains have been isolated from the human skin surface, oral cavity and genitourinary tract. Strains are rarely associated with acne vulgaris and some may be associated with skin health and others with opportunistic infection. The G+C content is ~ 60 % based on whole genome sequencing analysis.

The type strain of *Propionibacterium acnes* subspecies *defendens* subsp. nov. is ATCC11828 (=JCM 6473=CCUG 6369) isolated from a subcutaneous abscess (Genbank accession number NC_017550).

Description of *Propionibacterium acnes* subsp. *elongatum* subsp. nov.

Description for *Propionibacterium acnes* subsp. *elongatum* is given in Dekio et al. (2015).

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Table 1. Genetic characteristics of *P. acnes* phylogroups

Genetic Grouping	p-distance (core SNPs)			*ds**	% unique core region SNPs
	Type I	Type II	Type III		
Type I	-	0.444	0.487	0.006 ± 0.001	26
Type II	0.444	-	0.470	0.005 ± 0.001	22
Type III	0.487	0.470	-	0.002 ± 0.001	24
Type I, II, III	-	-	-	0.024 ± 0.003	-

*Based on the analysis of concatenated $MLSA_8$ sequence data using the Nei-Gojobori method (Jukes-Cantor) in MEGA v5.0.

Table 2. **Key phenotypic similarities and differences between type I, II and III strains**

Characteristic*	**Type I**	**Type II**	**Type III**
Indole production	+	d+	+
Catalase activity	+	+	+
Nitrate reduction	+	+	d+
Gelatin liquefaction	+	+	-
Aesculin Hydrolysis	-	-	-
β-haemolysis (5d at 37°C)	d+	-	-
Neuraminidase	d+	-	-
Lipase	d+	d-	d+
L-pyrrolydonyl arylamidase	d+	d-	-
Pyruvate	d+	+	-
Fermentation of:			
Sorbitol	d+	-	-
Maltose	-	-	-
Sucrose	-	-	-
Glycerol	d+	d+	+
Ribose	d-	d+	-
Cell wall components			
Dermatan sulphate-binding adhesins	d+	-	-
A_2pm isomer	LL-	LL- (*meso*)	ND
Amino acids	Ala, Gly, Glu	Ala, Gly, Glu	ND
Sugars	Galactose, Glucose, Mannose	Glucose Mannose	

+90% isolates are positive; -90% isolates are negative; d+40-89% isolates positive; d-11-39% isolates are positive.

*Key phenotypic characteristics were compiled from the data of one or more of the following publications: McDowell et al. (2005), McDowell et al. (2008), Lomholt and Kilian (2010), Niazi et al. (2010), McDowell et al. (2011), Patrick and McDowell (2011), Dekio et al. (2015).

Figure Legends.

Fig. 1. Minimum evolution phylogenetic tree (MEGA v7.0) (Kumar et al., 2016) of concatenated gene sequences (4253 bp) from all STs currently represented in the $MLST_8$ database (http://pubmlst.org/pacnes/), and covering all major genetic divisions**.** Sequence input order was randomized, and bootstrapping resampling statistics were performed using 500 data sets. Bootstrap values (≥70%) are shown on the arms of the tree. Horizontal bar represents genetic distance. CC= clonal complex.

Fig. 2. Neighbour-net split graph (SplitsTree v4.14.4) of allelic profiles from all STs currently represented in the $MLST_8$ database (http://pubmlst.org/pacnes/), and covering all major genetic divisions (Huson and Bryant, 2006). A distance matrix was generated from the allelic profile data and saved in NEXUS format for input to SplitsTree. Parallelogram formations indicative of recombination/ reticulation events are evident within the major type I and II divisions.

Figure 1

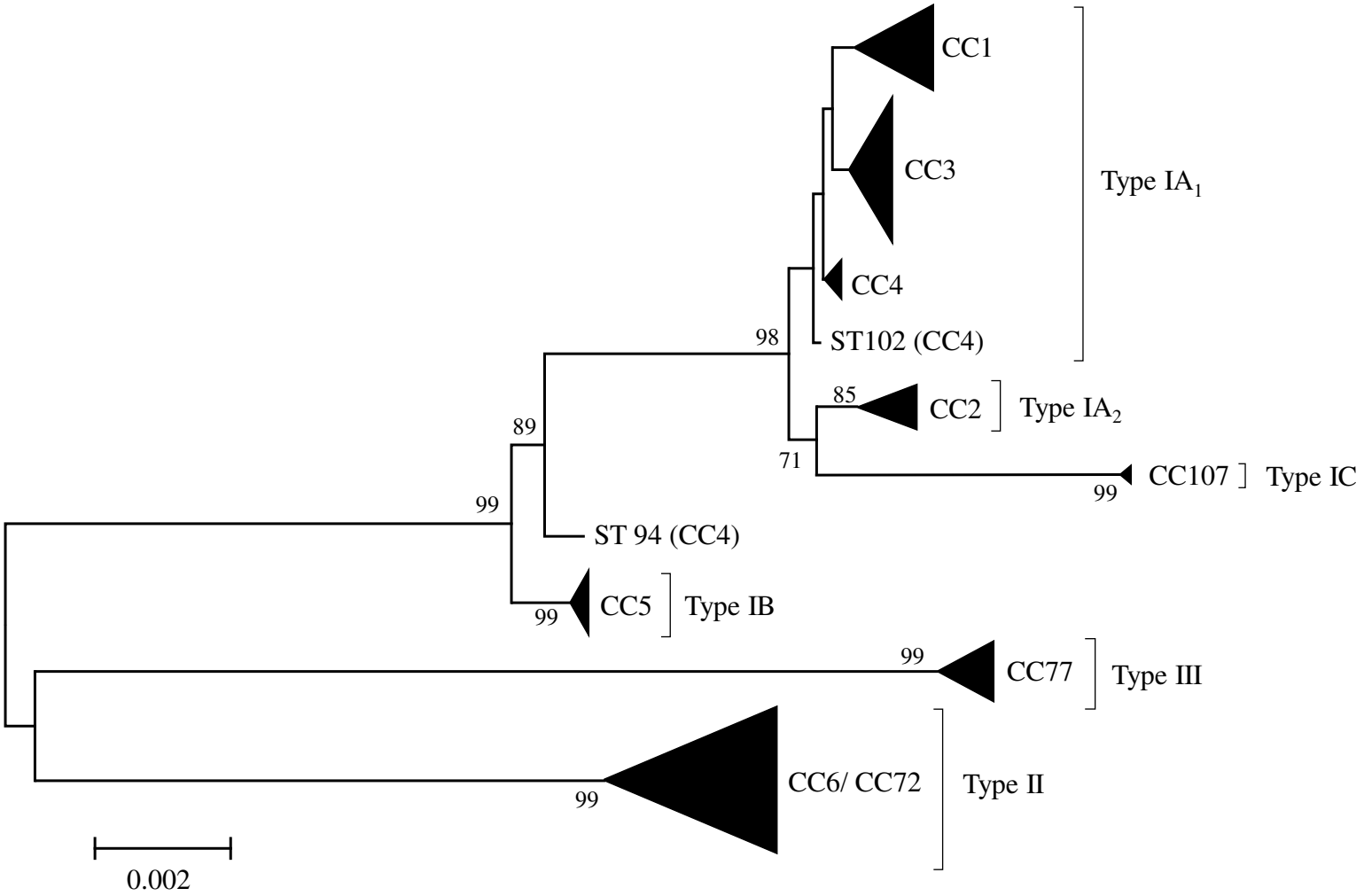
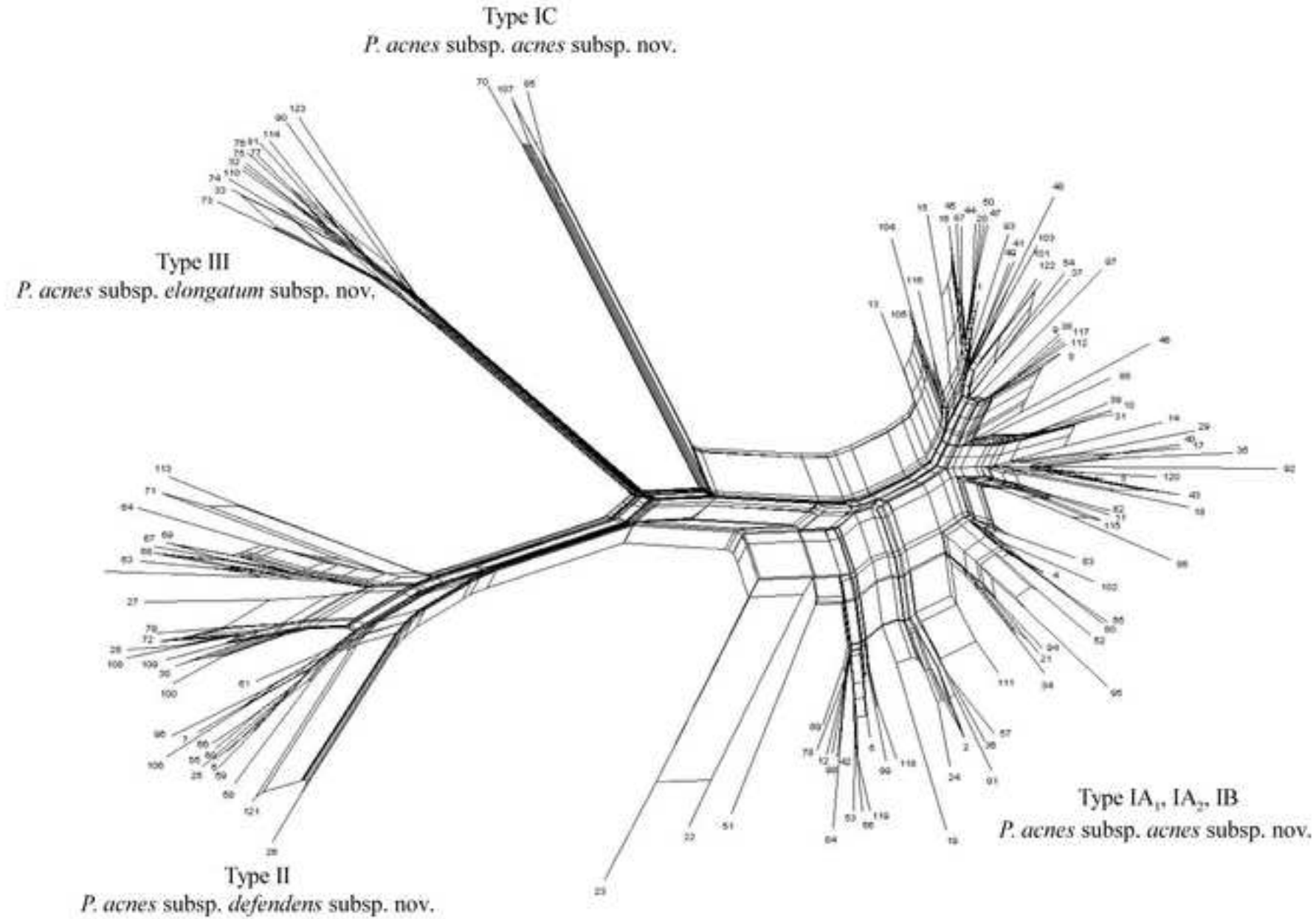


Figure 2



International Journal of Systematic and Evolutionary Microbiology
Supplementary Materials for:

Proposal to reclassify *Propionibacterium acnes* type I as *Propionibacterium acnes* subsp. *acnes* subsp. nov. and *Propionibacterium acnes* type II as *Propionibacterium acnes* subsp. *defendens* subsp. nov.

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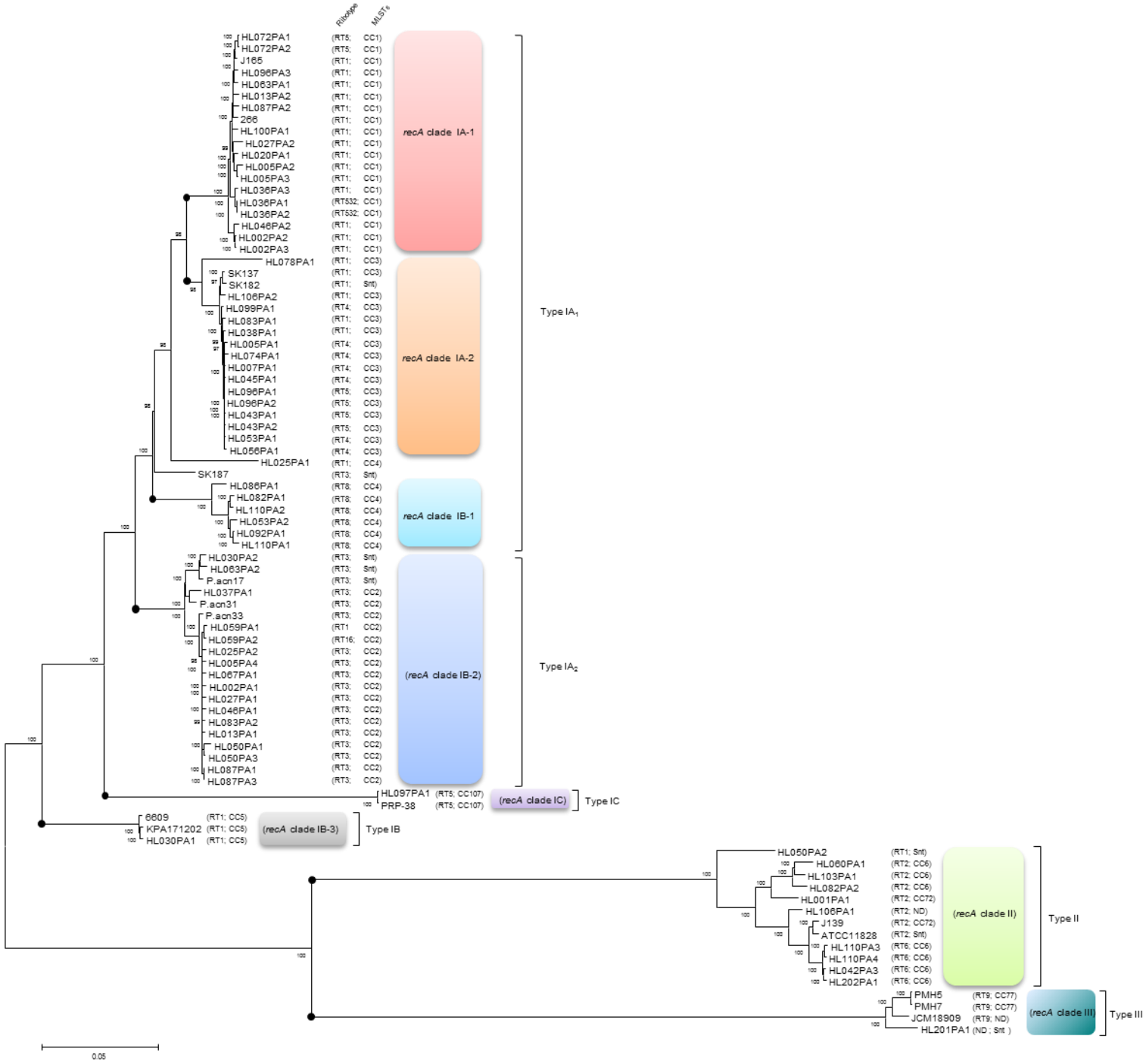
Ribotype
MLST
HL072PA1 (RT5; CC1)
HL072PA2 (RT5; CC1)
J165 (RT1; CC1)
HL096PA3 (RT1; CC1)
HL063PA1 (RT1; CC1)
HL013PA2 (RT1; CC1)
HL087PA2 (RT1; CC1)
266 (RT1; CC1)
HL100PA1 (RT1; CC1)
HL027PA2 (RT1; CC1)
HL020PA1 (RT1; CC1)
HL005PA2 (RT1; CC1)
HL005PA3 (RT1; CC1)
HL036PA3 (RT1; CC1)
HL036PA1 (RT532; CC1)
HL036PA2 (RT532; CC1)
HL046PA2 (RT1; CC1)
HL002PA2 (RT1; CC1)
HL002PA3 (RT1; CC1)
recA clade IA-1
HL078PA1 (RT1; CC3)
SK137 (RT1; CC3)
SK182 (RT1; Snt)
HL106PA2 (RT1; CC3)
HL099PA1 (RT4; CC3)
HL083PA1 (RT1; CC3)
HL038PA1 (RT1; CC3)
HL005PA1 (RT4; CC3)
HL074PA1 (RT4; CC3)
HL007PA1 (RT4; CC3)
HL045PA1 (RT4; CC3)
HL096PA1 (RT5; CC3)
HL096PA2 (RT5; CC3)
HL043PA1 (RT5; CC3)
HL043PA2 (RT5; CC3)
HL053PA1 (RT4; CC3)
HL056PA1 (RT4; CC3)
recA clade IA-2
HL025PA1 (RT1; CC4)
SK187 (RT3; Snt)
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HL082PA1 (RT8; CC4)
HL110PA2 (RT8; CC4)
HL053PA2 (RT8; CC4)
HL092PA1 (RT8; CC4)
HL110PA1 (RT8; CC4)
recA clade IB-1
Type IA1
HL030PA2 (RT3; Snt)
HL063PA2 (RT3; Snt)
P.acn17 (RT3; Snt)
HL037PA1 (RT3; CC2)
P.acn31 (RT3; CC2)
P.acn33 (RT3; CC2)
HL059PA1 (RT1 CC2)
HL059PA2 (RT16; CC2)
HL025PA2 (RT3; CC2)
HL005PA4 (RT3; CC2)
HL067PA1 (RT3; CC2)
HL002PA1 (RT3; CC2)
HL027PA1 (RT3; CC2)
HL046PA1 (RT3; CC2)
HL083PA2 (RT3; CC2)
HL013PA1 (RT3; CC2)
HL050PA1 (RT3; CC2)
HL050PA3 (RT3; CC2)
HL087PA1 (RT3; CC2)
HL087PA3 (RT3; CC2)
(recA clade IB-2)
Type IA2
HL097PA1 (RT5; CC107)
PRP-38 (RT5; CC107)
(recA clade IC)
Type IC
6609 (RT1; CC5)
KPA171202 (RT1; CC5)
HL030PA1 (RT1; CC5)
(recA clade IB-3)
Type IB
HL050PA2 (RT1; Snt)
HL060PA1 (RT2; CC6)
HL103PA1 (RT2; CC6)
HL082PA2 (RT2; CC6)
HL001PA1 (RT2; CC72)
HL106PA1 (RT2; ND)
J139 (RT2; CC72)
ATCC11828 (RT2; Snt)
HL110PA3 (RT6; CC6)
HL110PA4 (RT6; CC6)
HL042PA3 (RT6; CC6)
HL202PA1 (RT6; CC6)
(recA clade II)
Type II
PMH5 (RT9; CC77)
PMH7 (RT9; CC77)
JCM18909 (RT9; ND)
HL201PA1 (ND ; Snt)
(recA clade III)
Type III
0.05

Fig S1. Whole genome neighbour-joining phylogenetic tree (MEGA v5.0) based on the p-distances between each of 85 concatenated SNP sequences. Briefly, core regions were calculated by mapping 84 genome sequences against the reference genome KPA171202 using Nucmer [Kurtz et al. (2004). Versatile and open software for comparing large genomes. *Genome Biol* **5**: R12). Regions of KPA171202 that aligned with all 84 genomes were identified within the 84 ".coords" output files, and the corresponding aligned regions in all 85 genomes were extracted. A total of 124,731 SNPs relative to KPA171202 were identified and concatenated into a single sequence for analysis. The bootstrap tree was inferred from 200 replicates. Bootstrap values (≥70%) are shown on the arms of the tree. Horizontal bar represents genetic distance. Snt = singleton; CC = clonal complex ($MLST_8$ analysis).

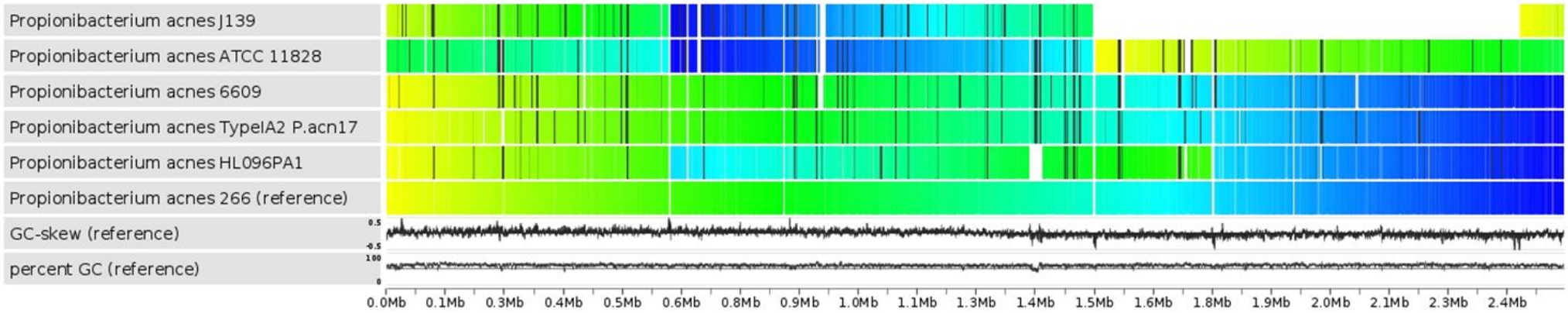

Fig S2. Visualisation of synteny between representative type I and type II genomes using the new *P. acnes* Sybil database. Type II specific inversions within the strains ATCC11828 (ST27) and J139 (ST28) can be clearly seen versus type I strains 266 (IA_1; ST20), P.acn17 (IA_2; ST22) and 6609 (IB; ST5). Note: the type IA_1 strain HL096PA1 (ST3) has a distinct verified inversion while those in the type II strains remained to be confirmed. Strain J139 is currently not closed and is represented by a large contig which results in a white section in the alignment [Scholz et al. (2016). Genome stability of *Propionibacterium acnes*: a comprehensive study of indels and homopolymeric tracts. *Sci Rep,* **6,** 20662]. ST designations relate to $MLST_8$ analysis (http://pubmlst.org/pacnes/).